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Capnocytophaga canimorsus sp. nov. (Formerly CDC Group DF-2), a Cause of Septicemia following Dog Bite, and *C. cynodegmi* sp. nov., a Cause of Localized Wound Infection following Dog Bite

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CDC group DF-2 is the vernacular name given to a slow-growing gram-negative bacterium that causes septicemia and meningitis in humans. Infections frequently (one-third of cases) occur following dog bites or close contact with dogs or occasionally with cats. Splenectomy and alcoholism appear to be strong predisposing factors for DF-2 infection. In addition to 150 DF-2 strains received for identification, we received 9 DF-2-like strains; 6 were isolated from wound or eye infections, 3 of which were associated with dog bites and 1 of which was associated with a cat scratch, and 3 were isolated from dog mouths. The major characteristics of DF-2 include production of acid but no gas from lactose and maltose and usually D-glucose; positive reactions for oxidase, catalase, arginine dihydrolase, gliding motility, and *o*-nitrophenyl-β-D-galactopyranoside; growth enhanced by serum and by incubation in a candle jar atmosphere; and negative reactions for sucrose, raffinose, inulin, melibiose, nitrate reduction, indole, and growth on MacConkey agar. DF-2-like strains had the same characteristics, except that acid was formed from sucrose, raffinose, inulin, and melibiose. By the hydroxyapatite method, DNAs from 12 DF-2 strains were 88% related in 60°C reactions and 84% related in 75°C reactions. Related sequences contained 0.5 to 1.5% unpaired bases (divergence). Three DF-2-like strains were 73 to 80% related at 60°C (with 2.0 to 2.5% divergence) and 68 to 75% related at 75°C. The relatedness of DF-2 and DF-2-like strains was 19 to 31% at 60°C and 13 to 19% at 75°C. The relatedness of DF-2 and DF-2-like strains to *Capnocytophaga* species was 4 to 7%. The DNA relatedness data indicate that the DF-2 and the DF-2-like strains are separate, previously undescribed species. Both groups are phenotypically and genetically distinct from *Capnocytophaga* species, although they do share several characteristics with *Capnocytophaga* species, including cellular morphology, gliding motility, cellular fatty acid composition, enhancement of growth in a candle jar atmosphere, and G+C content. The new species differ from *Capnocytophaga* species by their positive oxidase and catalase reactions. We chose to avoid creating a new genus and proposed the names *Capnocytophaga canimorsus* sp. nov. for group DF-2 and *C. cynodegmi* sp. nov. for the DF-2-like strains.

The vernacular name CDC group DF-2 was given to an unclassified group of slow-growing, gram-negative, fermentative bacteria that were referred for identification to the Centers for Disease Control. DF stands for dysgonic (slow and relatively poor growth of a bacterial culture) fermenter. The first DF-2 strain was received at the Centers for Disease Control in 1961.

A case report of a "previously undescribed gram-negative bacillus causing septicemia and meningitis" appeared in 1976 (1). Shortly thereafter, Butler et al. reviewed clinical and epidemiologic findings for 17 patients from whose blood DF-2 strains were isolated (3). All of the patients had fever and septicemia, seven had associated cellulitis, four had meningitis, and three had endocarditis; three patients died. Ten of the patients had a history of recent dog bites, and four others had contact with dogs. Underlying diseases were also important. Only two of the patients had previously been in good health. Five had undergone splenectomy, and four had a history of alcoholism.

Hicklin et al. recently reviewed the literature on 42 group DF-2 cases, including 1 they reported (8). Twenty-three patients had been bitten by dogs, and a total of 35 had recent contact with dogs (including one exposure to coyotes). Only 4 patients were in good health before DF-2 infection; 16 had undergone splenectomy, 12 had alcoholism, and 2 were receiving corticosteroids. Eleven deaths were reported. Carpenter et al. reported two cases of DF-2 bacteremia following cat bites (4).

Through 1987, 150 DF-2 strains were received for identification in the Special Bacteriology Reference Laboratory in the Meningitis and Special Pathogens Branch at the Centers for Disease Control. DF-2 is easily differentiated from other fastidious gram-negative bacteria that occur in blood cultures, e.g., *Haemophilus aphrophilus*, *Actinobacillus* (*Haemophilus*) *actinomycetemcomitans*, *Cardiobacterium hominis*, *Campylobacter* species, and *Brucella* species (5). A second group of strains isolated from dogs or noninvasive wound infections caused by dog bites consisted of eight strains that differed from DF-2 in their ability to ferment sucrose, raffinose, melibiose, and inulin and one strain that differed from DF-2 only by melibiose fermentation and marked adherence to blood agar plates (this characteristic was lost during storage). These nine strains were termed DF-2-like.

The purpose of this study was to examine DF-2 and DF-2-like strains phenotypically and by DNA hybridization to determine whether these strains represented one or more

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TABLE 1. Sources and DNA relatedness of DF-2 and DF-2-like strains

Strain[a]	Source of isolation	Received from	DF-2 7120[T]			DF-2-like E6447[T]			DF-2-like F1750		
			RBR[b], 60°C	D[c]	RBR, 75°C	RBR, 60°C	D	RBR, 75°C	RBR, 60°C	D	RBR, 75°C
DF-2											
7120[T] (ATCC 35979)	Human blood	California SHD[d]	100	0.0	100	23		16	19		13
A3565	Human blood	Tennessee SHD	85	1.0	77						
B5831	Human blood	Washington SHD	91	0.0	84						
B6953	Human blood	California SHD	97	1.0	90				21		14
C3433	Human blood	Minnesota SHD	90	0.5	87	24		16			
C3556	Human blood	Indiana SHD	86	1.0	81						
C6704	Human blood	Tennessee SHD	87	0.5	84						
C7423	Human blood	Washington SHD	91	0.5	89						
D4689	Human blood	New York SHD	87	0.0	85						
D8872	Human blood	Utah SHD	85	0.0	84						
E6231	Human blood	Virginia SHD	89	0.0	90						
F3471	Human blood	Georgia SHD	92[e]	1.5[e]							
DF-2-like											
E679	Hand wound	Iowa SHD	31[e]						79	2.5	68
E6447[T] (=ATCC 49044)	Dog mouth	Virginia SHD	28		21	100	0.0	100	73	2.5	69
F1750 (=ATCC 49045)	Hand wound	Missouri SHD	25		18	80	2.0	75	100	0.0	100
Capnocytophaga gingivalis ATCC 33624[T]			7						4		
Capnocytophaga ochracea ATCC 33596[T]			6								
Capnocytophaga sputigena ATCC 33612[T]			6								
Cardiobacterium hominis 6573A			1			1					

[a] This strain column also indicates the source of unlabeled DNA. ATCC, American Type Culture Collection.
[b] RBR, Relative binding index (% relatedness).
[c] D, Divergence (unpaired bases) in related sequences. Calculated to the nearest 0.5%.
[d] SHD, State Health Department.
[e] Data obtained by using labeled DNA from strain B6953.

groups, to assess any differences in pathogenicity, and to properly classify them.

MATERIALS AND METHODS

Bacterial strains. All of the strains were grown on plates containing heart infusion agar with 5% rabbit blood. Incubation was at 35°C for 24 h in a candle extinction jar. The strains used in DNA hybridization studies are listed in Table 1.

DNA tests. G+C content was determined spectrophotometrically by thermal denaturation (11). The preparation and purification of DNA and the conditions used to determine DNA relatedness by the hydroxyapatite method have been previously described (2). DNAs from DF-2 and DF-2-like strains were labeled in vitro with [^{32}P]dCTP, provided in a nick translation reagent kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.).

Biochemical tests. Biochemical tests were done as previously described (5). Decarboxylase tests were performed with heavy inocula by the method described for nonfermentative bacteria (5). Gliding motility was determined by inoculating cultures with a loop to the surface of heart infusion agar containing 5% rabbit serum and incubating them for 24 h at 35°C in a candle extinction jar. Observations were made at room temperature by covering a section of the margin of bacterial growth with a cover slip and examining it by bright-field microscopy with a ×100 oil immersion lens (magnification, about ×1,000). Gliding motility was recorded as positive when individual cells, within a field in which most of the cells were stationary, were observed to move a distance of 1.5 to 2 or more cell lengths. Some cells were usually observed to move this distance within 60 s. Movement of cells of strain 7120 was documented by photographing a single microscopic field five times at 10-s intervals. Carbohydrate fermentation tests were done by the following four methods: (i) addition of 1 drop of broth culture to 3 ml of enteric fermentation base broth contained in tubes (15 by 125 mm); (ii) same as above, with 2 drops of rabbit serum added to the broth; (iii) 0.3 ml of enteric fermentation base in tubes (13 by 100 mm) inoculated with 2 drops of a heavy (milky) suspension obtained from a 24- to 48-h culture suspended in enteric fermentation base without carbohydrate; (iv) rapid fermentation test (read after 4 h of incubation) in which a heavy inoculum was added to a small volume of concentrated carbohydrate in buffer and phenol red (5).

RESULTS AND DISCUSSION

Source, incidence, and clinical significance. The culture collection of the Special Bacteriology Reference Laboratory at the Centers for Disease Control includes 150 DF-2 and 9 DF-2-like strains. One hundred thirty-two DF-2 strains (88%) were isolated from human blood, seven (4.7%) were from cerebrospinal fluid (six of the isolates from blood were also isolated from cerebrospinal fluid), three (2%) each were from wounds and dog mouths, and one each was from a heart valve, petechiae, a cornea, an adrenal gland, and an

unknown source. All isolates from blood and cerebrospinal fluid were from patients diagnosed as septicemic with fever.

Eighty-four isolates of DF-2 strains from humans and two from dogs were chosen for further study. They were received from 31 of the United States, Australia, Canada, Denmark, England, France, The Netherlands, New Zealand, Sweden, and The Union of South Africa. Among the patients whose sexes were known, 53 (74%) of 72 strains were isolated from men. Age was reported for 66 patients. Of these, 3% were <20 years old, 8% were 20 to 39 years old, 8% were 30 to 39 years old, 15% were 40 to 49 years old, 23% were 50 to 59 years old, 30% were 60 to 69 years old, and 14% were 70 years old or older. Thirty-six (43%) of the patients reported having been bitten or scratched by dogs or, in two cases, having been bitten or scratched by cats. Another 10 patients reported exposure to dogs, 1 reported a cut contaminated with rabbit feces, and 1 patient was an animal photographer at a zoo. Splenectomy was a predisposing factor in 18 cases (21%), alcoholism in 6 cases (7%), and renal transplant in 1 case. There were three reported fatalities. This incidence is somewhat lower than those of dog and cat bites (55%), splenectomies (38%), alcoholism (29%), and fatalities (26%) found in previous case studies (8). In all probability, these factors are underreported in the present study because no attempt was made to obtain clinical data in addition to those voluntarily submitted with the DF-2 cultures.

The DF-2-like strains were isolated from human wound infections (five strains; 56%), dog mouths (3 strains; 33%), and a patient with endophthalmitis following a corneal transplant. Three of six human wound infections resulted from dog bites, and one was from a cat scratch. There was no evidence of systemic infection caused by DF-2-like strains. The DF-2-like strains isolated from humans were received from Pennsylvania (two strains), Iowa, Maryland, Missouri, and Oklahoma; the strains isolated from dogs were received from Ohio (two strains) and Virginia.

Thus, both DF-2 and DF-2-like strains cause human infections, often by way of dog or cat bites or scratches. Both DF-2 and DF-2-like strains have been isolated from the mouths of apparently healthy dogs. Human isolates of DF-2-like strains usually are associated with localized wound infections, whereas DF-2 strains are more frequently isolated from patients with septicemia, often in individuals seemingly predisposed to infection by splenectomy or alcoholism.

DNA studies. DNAs from DF-2 strains 7120^T, B6953, C3433, and D4689 each had a G+C content of 36 mol%. DNAs from DF-2-like strains E6447^T and F1750 each had a G+C content of 34 mol%.

DNAs from DF-2 and DF-2-like strains were labeled for use in DNA hybridization. All of the DF-2 strains tested were at least 85% related to labeled DNA from DF-2 strain 7120^T in 60°C reactions and at least 77% related to it in 75°C reactions (Table 1). Divergence (unpaired bases) in related sequences was 0.0 to 1.5% (Table 1). Similar results (88% average relatedness at 60°C; 1.5% average divergence) were obtained with labeled DNA from DF-2 strain B6953 (data not shown). Three DF-2-like strains were 28% related to DF-2 strain 7120^T, and the relatedness of DF-2 to *Capnocytophaga* species and *Cardiobacterium hominis* was 7% or less (Table 1).

The three DF-2-like strains tested were 73 to 80% related to labeled DF-2-like DNA at 60°C, with 2.0 to 2.5% divergence in related sequences and 68 to 75% relatedness at 75°C (Table 1). Labeled DF-2 strains were 19 to 24% related to labeled DF-2-like DNAs, and relatedness of DF-2-like DNA to *Capnocytophaga gingivalis* and *Cardiobacterium hominis* was 4% or less.

The DF-2 and DF-2-like DNA relatedness groups were distinct from one another and from their closest phenotypic relatives in the genera *Capnocytophaga* and *Cardiobacterium*. Each group, therefore, represents a single, previously undescribed species for which names are proposed and descriptions are given below.

Biochemical characterization. Growth of DF-2 and DF-2-like strains was enhanced by addition of rabbit serum and incubation in a candle jar. In comparison with heart infusion agar, tryptic soy agar was a poor growth medium for DF-2 and DF-2-like organisms. Tryptic soy blood agar plates should therefore not be used to culture dog bite wounds or to subculture blood culture bottles if these organisms are suspected. The biochemical reactions of DF-2 and DF-2-like strains are shown in Table 2. Reactions useful for differentiating DF-2 and DF-2-like strains from one another and from *Capnocytophaga* species are shown in Table 3. DF-2 strains did not ferment inulin, melibiose (6% positive), raffinose, and sucrose. All DF-2-like strains fermented melibiose, but one strain failed to ferment inulin, raffinose, and sucrose. This atypical strain was also unique in showing marked adherence to the surface of blood agar. This characteristic was lost during storage.

Taxonomic description. The genus *Capnocytophaga*, with the species *C. ochracea*, *C. gingivalis*, and *C. sputigena*, was proposed in 1979 (9, 10, 12, 15) for gram-negative, oxidase-negative, catalase-negative, fermentative, microaerophilic bacteria that exhibited gliding motility. *Capnocytophaga* species were differentiated by a combination of carbohydrate fermentation and hydrolysis reactions, none of which were definitive (12). More recent studies (13) indicated that *C. gingivalis*, the least metabolically active species, could be identified biochemically, but *C. ochracea* and *C. sputigena* were not separable from one another except by DNA hybridization. Cell wall fatty acids and isoprenoid quinone composition are essentially identical in the three *Capnocytophaga* species (6, 7, 13). In DNA hybridization reactions, *C. ochracea* and *C. sputigena* were 36% interrelated, and each species showed about 12% relatedness to *C. gingivalis*.

DF-2 and DF-2-like strains differ from *Capnocytophaga* species by their positive oxidase and catalase reactions but resemble *Capnocytophaga* species in other characteristics. Their growth is stimulated by addition of serum to the medium and although they are not obligately microaerophilic, growth is substantially better in a CO_2-enriched atmosphere. They exhibit gliding motility and are fermentative, and their cell wall fatty acids are indistinguishable from those of *Capnocytophaga* species (7; C. W. Moss, personal communication). Their DNA relatedness to *Capnocytophaga* species is 4 to 7%, similar to the values obtained by Speck et al. (13). One could create a new genus of oxidase- and catalase-positive gliding bacteria for DF-2 and DF-2-like strains, but we prefer to place them in *Capnocytophaga* on the basis of their overall phenotypic similarity to this genus. Furthermore, placing DF-2 and DF-2-like strains in *Capnocytophaga* does not appear to add to the genetic divergence already present in this genus.

Description of *Capnocytophaga canimorsus* sp. nov. *C. canimorsus* (ca.ni.mor'sus. L. *canis*, dog; L. *morsus*; a bite, biting; N.L. gen. n. *canimorsus* of a dog bite) is proposed for group DF-2. Cells are thin, gram-negative, nonsporeforming rods 1 to 3 μm long. In preparations for blood agar, longer

TABLE 2. Biochemical characteristics of DF-2 and DF-2-like strains at 48 h (7 days)

Characteristic (no. of DF-2 and DF-2-like strains)	% of DF-2 strains positive at 48 h (7 days)	Result[a] with strain 7120[T]	% of DF-2-like strains positive at 48 h (7 days)	Result[a] with strain E6447[T]
Oxidase (74; 9)	97	+	100	+
Catalase (75; 8)	100	+	100	+
MacConkey agar (growth) (68; 9)	2	−	0	−
Citrate (Simmons) (67; 9)	0	−	0	−
Urease (Christensens) (62; 9)	0	−	0	−
Nitrate reduction to nitrite (74; 6)	0	−	17	−
Nitrite reduction (5; 6)	71	+	67	+
Indole production (58; 9)	0	−	0	−
Lysine decarboxylase (65; 9)	0	−	0	−
Arginine dihydrolase (69; 9)	99	+	100	+
Ornithine decarboxylase (65; 9)	0	−	0	−
o-Nitrophenyl-β-D-galactopyranoside (63; 8)	100	+	100	+
Esculin hydrolysis (72; 9)	28 (64)	+	78 (100)	(+)
Gelatin hydrolysis (49; 3)	0	−	0	−
Gliding motility (11; 3)	100	+	100	+
Gas from D-glucose (41; 9)	0	−	0	−
Acid from[b]:				
Cellobiose (18; 3)	28 (39)	−	33 (100)	(+)
Dextrin (18; 3)	83	+	100	+
Fructose (18; 3)	33 (44)	−	100	+
D-Galactose (18; 3)	89	+	67	+
D-Glucose (41; 9)	81 (93)	+	100	+
Glycogen (18; 3)	89	+	33 (100)	+
Inulin (25; 9)	0	−	89	+
Lactose (40; 9)	90 (100)	+	100	+
Maltose (40; 9)	80 (95)	+	100	+
D-Mannitol (40; 9)	0	−	0	−
D-Mannose (12; 3)	83	+	100	+
Melibiose (18; 3)	6	−	100	+
Raffinose (35; 9)	0	−	89	+
Starch (17; 3)	88	+	100	+
Sucrose (40; 9)	0	−	89	+
D-Xylose (40; 9)	0	−	0	−

[a] +, positive at 48 h; (+), positive at 7 days; −, negative at 7 days.
[b] Small-volume fermentation method.

rods, filaments (often curved), spindle-shaped cells, and coccoid forms are also present. Flagella are not present; gliding motility as seen by bright-field microscopy. Colonies on blood agar at 18 to 24 h are less than 0.5 mm in diameter and may be either convex or flat. Flat colonies usually are irregular in shape. Some convex colonies have a very narrow, flat edge. After incubation for 48 h, colonies are 1 to 3.5 mm in diameter. The surface is raised or slightly uneven, and there may or may not be a flat, spreading edge. No hemolysis occurs on rabbit blood agar, but confluent growth is seen at 24 h, and colonies at 48 h frequently appear to be light purple. The cell mass, when removed from the plate on a loop, however, is light yellow. No growth on MacConkey agar. No growth or a neutral reaction on triple sugar iron agar. Microaerophilic, grow best on heart infusion agar supplemented with 5% rabbit or sheep blood incubated at 35 to 37°C in the presence of CO_2 (candle extinction jar).

TABLE 3. Differentiation of DF-2 and DF-2-like strains

Characteristic	% Positive at 48 h (7 days)[a]		Result[b] with:		
	DF-2 strains	DF-2-like strains	*C. gingivalis*	*C. ochracea*	*C. sputigena*
Oxidase	97	100	−	−	−
Catalase	100	100	−	−	−
Fructose	33 (44)	100	(+)	(+)	(+)
Glycogen	89	33 (100)	+	+	−
Inulin	0	89	+	+	+
Melibiose	6	100	−	−	−
Raffinose	0	89	+	+	+
Sucrose	0	89	+	+	+

[a] These data are from Table 2.
[b] +, ≥90% positive at 48 h; (+), ≥90% positive at 7 days; −, <10% positive at 7 days.

Positive reactions in tests for oxidase (some strains are weakly positive), catalase, arginine dihydrolase, *o*-nitrophenyl-β-D-galactopyranoside, and alkaline phosphatase (13). Acid production from D-glucose, lactose, and maltose and in most strains from dextrin, D-galactose, glycogen, D-mannose, and starch. Variable reactions (10 to 75% positive) in tests for nitrite reduction, esculin hydrolysis, and acid production from cellobiose and fructose. Negative reactions in tests for indole production, growth on citrate, H_2S production (13), urease, nitrate reduction, lysine and ornithine decarboxylases, gelatin hydrolysis, gas production from D-glucose, and acid production from adonitol (13), L-arabinose (13), dulcitol (13), *meso*-inositol (13), inulin, D-mannitol, melibiose, raftinose, L-rhamnose (13), salicin (13), sorbitol (13), L-sorbose (13), sucrose, trehalose (13), and D-xylose.

Butler et al. reported that DF-2 is susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, clindamycin, erythromycin, penicillin, and tetracycline; resistant to colistin, gentamicin, and kanamycin; and variably resistant to trimethoprim-sulfamethoxazole (3). In contrast, Verghese et al. (14) recently tested nine strains and found them to be susceptible to trimethoprim-sulfamethoxazole and aminoglycosides; colistin was not tested.

The G+C contents of DNAs from the type strain and three other strains were 36 mol%.

Isolated from human blood, cerebrospinal fluid, and wounds and from the mouths of healthy dogs. Pathogenic for humans, causing septicemia, especially in individuals predisposed by splenectomy or alcoholism. The route of infection is through the skin after a bite or scratch from a dog or, rarely, a cat or other animal. The route of infection was unknown in 20% of the cases reviewed by Hicklin et al. (8).

The type strain is 7120 (=ATCC 35979), which was isolated in Upland, Calif., from the blood of a 17-year-old male in 1961 (3).

Biochemical tests to differentiate *C. canimorsus* from other *Capnocytophaga* species are shown in Table 3.

Description of *Capnocytophaga cynodegmi* sp. nov. *C. cynodegmi* (sy.no.deg'mi. Gr. *kyon*, *kyno-*, dog; Gr. *degmos*, a bite; N.L. gen. n. *cynodegmi* of a dog bite) is proposed for group DF-2-like organisms. Cells are thin, gram-negative, nonsporeforming rods 1 to 3 μm long. Longer rods and filaments, often curved, are also present. Flagella are not present; gliding motility as seen by bright-field microscopy. Colonies on blood agar at 18 to 24 h are either punctate (less than 0.5 mm in diameter) and convex or flat and irregular in shape with a diameter of 0.5 to 1 mm. After incubation for 48 h, colonies are 3 to 4 mm or larger in diameter. The surface is smooth or slightly uneven. Some colonies have a narrow, flat, spreading edge. Five of nine strains produced beta-like hemolysis in rabbit blood agar, best demonstrated by stabbing an inoculum into the agar. Confluent growth at 24 h, and colonies at 48 h frequently appeared to be light purple. The cell mass when removed from the plate on a loop, however, was light yellow. No growth on MacConkey agar; no growth or a neutral reaction on triple sugar iron agar. Microaerophilic, grows best on heart infusion agar supplemented with 5% rabbit or sheep blood incubated at 35 to 75°C in the presence of CO_2 (candle extinction jar).

Positive reactions in tests for oxidase (some strains were weakly positive), catalase, arginine dihydrolase, *o*-nitrophenyl-β-D-galactopyranoside, esculin hydrolysis (delayed), and acid production from cellobiose (delayed), dextrin, fructose, D-glucose, glycogen (delayed), lactose, maltose, D-mannose, melibiose, and starch and in most strains from inulin, raffinose, and sucrose. Variable reactions (10 to 75% positive) in tests for nitrate reduction, nitrite reduction, and acid production from D-galactose. Negative reactions in tests for indole production, growth on citrate, urease, lysine and ornithine decarboxylases, gelatin hydrolysis, gas from D-glucose, and acid production from D-mannitol and D-xylose.

The G+C contents of DNAs from the type strain and another strain were 34 mol%.

Isolated from human wounds and mouths of apparently healthy dogs. Pathogenic for humans, causing wound infections. The route of infection is through the skin after a dog bite or, in one case, a cat scratch. No evidence of systemic infection or predisposing factors.

The type strain is E6447 (=ATCC 49044), isolated in Virginia from the mouth of a dog in 1979.

Biochemical tests to differentiate *C. cynodegmi* from other *Capnocytophaga* species are shown in Table 3.

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